Contents lists available at ScienceDirect



Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc



Keita Matsuno^a, Eri Nakayama^a, Osamu Noyori^a, Andrea Marzi^b, Hideki Ebihara^b, Tatsuro Irimura^c, Heinz Feldmann^b, Ayato Takada^{a,*}

^a Department of Global Epidemiology, Hokkaido University Research Center for Zoonosis Control, Sapporo, Japan

^b Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rocky Mountain Laboratories,

Hamilton, MT, USA

^c Graduate School of Pharmaceutical Science, University of Tokyo, Tokyo, Japan

ARTICLE INFO

Article history: Received 21 October 2010 Available online 5 November 2010

Keywords: Filovirus Virus entry C-type lectin

ABSTRACT

Cellular C-type lectins have been reported to facilitate filovirus infection by binding to glycans on filovirus glycoprotein (GP). However, it is not clearly known whether interaction between C-type lectins and GP mediates all the steps of virus entry (i.e., attachment, internalization, and membrane fusion). In this study, we generated vesicular stomatitis viruses pseudotyped with mutant GPs that have impaired structures of the putative receptor binding regions and thus reduced ability to infect the monkey kidney cells that are routinely used for virus propagation. We found that infectivities of viruses with the mutant GPs dropped in C-type lectin-expressing cells, parallel with those in the monkey kidney cells, whereas binding activities of these GPs to the C-type lectins were not correlated with the reduced infectivities. These results suggest that C-type lectin-mediated entry of filoviruses requires other cellular molecule(s) that may be involved in virion internalization or membrane fusion.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Ebola virus (EBOV) and Marburg virus (MARV) are enveloped negative-strand RNA viruses that constitute the family *Filoviridae*. Filovirus infection causes severe hemorrhagic fever in humans and non-human primates and mortality rates have ranged up to 90%. *Zaire ebolavirus* (ZEBOV) has caused multiple large outbreaks with the highest mortality rates (~90%) among EBOV species. Among MARVs, strain Angola (MARV-A) caused the largest outbreak in 2004–05 in Angola, with the highest mortality rate (90%) [1].

It has been shown that the filovirus entry into host cells depends on endosomal acidification [2,3] and proteolysis of the glycoprotein (GP) by endosomal cysteine proteases like cathepsin B and/or L [4]. Filovirus GP is the only spike protein on the surface of the virion, and therefore GP is responsible for both receptor binding and membrane fusion. GP is comprised of two molecules, GP1 and GP2, which are linked by a disulfide bond. GP1 contains a putative receptor binding region (RBR) [5,6] and a mucin-like region (MLR) that has a number of potential N- and O-linked glycosylation sites [7,8]. GP2 has a transmembrane domain, cytoplasminc tail and an internal fusion loop [1].

GP1, in particular MLR, is highly glycosylated by both N- and O-glycans, and these glycans are thought to be recognized by

* Corresponding author. Fax: +81 11 706 7310.

E-mail address: atakada@czc.hokudai.ac.jp (A. Takada).

cellular C-type lectins such as liver-specific C-type lectin asialoglycoprotein receptor (ASGP-R) [9,10], dendritic cell- and liver/lymph node-specific ICAM-3-grabbing nonintegrin (DC-SIGN and L-SIGN) [10–18], human macrophage galactose-type C-type lectin (hMGL) [18,19], and liver and lymph node sinusoidal endothelial cell Ctype lectin (LSECtin) [12,17]. Though these C-type lectins show different specificities, depending on the structures of target glycans, all have been reported to promote filovirus entry. Hepatocytes, dendritic cells, monocytes and macrophages are thought to be the preferred target cells of filoviruses, and infection of these cells is important for hemorrhagic manifestation and immune disorders [20–23]. Thus, increased infection of these cells might be directly involved in the pathogenesis of filovirus infection [18,24].

Though the C-type lectins have been reported to enhance filovirus infection, DC-SIGN and L-SIGN did not confer susceptibility for EBOV to non-susceptible cells, i.e. CD4+T-cells [11] and Ramons B cells [14]. In readily susceptible cells, it was reported that the internalization of DC-SIGN and L-SIGN themselves was not essential for trafficking EBOV into endosomal compartments [14]. These studies suggest that C-type lectins promote the filovirus entry by enhancing the virion attachment on the cell surface but not by enhancing the virion internalization. However, it has not been clarified yet whether C-type lectins independently act as a functional receptor mediating all the steps of viral entry including attachment, internalization, and membrane fusion. In the present study, to confirm the role of the C-type lectins in filovirus entry, we generated mutant GPs whose RBRs were impaired, and examined their abilities

⁰⁰⁰⁶⁻²⁹¹X/\$ - see front matter \circledcirc 2010 Elsevier Inc. All rights reserved. doi:10.1016/j.bbrc.2010.10.136



Fig. 1. Infectivity of VSVAG* pseudotyped with GPARBR. Functional domains and putative regions of ZEBOV GP and A-MARV GP are represented in schematic forms (A) (SP; signal peptide, RBR; receptor binding region, MLR; mucin-like region, IFL; internal fusion loop, and TM; transmembrane domain). Infectivities of the viruses in Vero E6, 293, HepG2, K562/mock, K562/hMGL, and K562/DC-SIGN were determined by counting GFP-positive cells and the infectious units (IUs) are indicated on the vertical lines (B). All experiments were done at least three times and averages and standard deviations are shown.

to infect C-type lectin-expressing cells without the interaction between RBR and its unknown putative counterpart(s).

2. Materials and methods

2.1. Cells

293T, Vero E6, and HEK293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics. HepG2 cells were grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics. K562 cell clones expressing hMGL (K562/hMGL), DC-SIGN (K562/DC-SIGN), and mock transfected (K562/mock) were grown in RPMI 1640 supplemented with 10% fetal bovine serum, L-glutamine, and antibiotics.

2.2. Viruses

Construction of mutant GPs was done as previously described [18]. The modified GP genes were then ligated into pCAGGS and used to express GPs on 293T cells. Vesicular stomatitis virus expressing green fluorescent protein (GFP) (VSV ΔG^*) pseudotyped with GP was generated in 293T cells as previously described [2,18].

 Table 1

 Characteristics of entry deficient mutant GPs.

ZEBOV CP +++++ ZEBOV CPA54-149 ++ ZEBOV CPA54-27 ++++ ZEBOV CPA54-313 ND ND ND ZEBOV CPA54-313 ND ZEBOV CPA54-313 ND ZEBOV CPA89-227 ++++ TEBOV CPA89-313 ND D55A ++++ TSTA ++++ [27] L57A ++++ [27] L57A ++++ [27] L57A ++++ [27] L57K ++++ [27] L57K ++++ [27] L57K ++++ [27] L57K ++++ [27] R64E ++++ [27] R64E ++++ [27] R134A ++++ [27] R134A ++++ [27] K140A ++++ [27] MARV-A GP +++++ [27] </th <th></th> <th>Protein expression^a</th> <th>Virion incorporation^b</th> <th>Reference</th>		Protein expression ^a	Virion incorporation ^b	Reference
ZEBOV GP ++++ +++++ ZEBOV GPA54-149 ++ + ZEBOV GPA54-227 ++++ +++ ZEBOV GPA54-313 ND ND ZEBOV GPA89-149 ++ + ZEBOV GPA89-227 ++++ ++++ ZEBOV GPA89-313 ND ND D55A ++++ ++++ [27] L57A ++++ L57I ++++ ++++ [27] L57K ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R134A ++++ [27] R134A ++++ [27] R134A ++++ [29] G143A ++++ [29] I170A ++++ [27] MARV-A GPA38- ND ND 308		скрісэзіон	incorporation	
ZEBOV GPA54-149 ++ + ZEBOV GPA54-227 ++++ +++ ZEBOV GPA54-313 ND ND ZEBOV GPA89-149 ++ + ZEBOV GPA89-227 ++++ ++++ ZEBOV GPA89-313 ND ND D55A ++++ ++++ [27] L57A ++++ L57F ++++ ++++ [27] L57K ++++ L57K ++++ ++++ [27] L63A ++++ [27] L63A ++++ [27] L63A ++++ [27] L63A ++++ [27] R64E ++++ [27] R64E ++++ [27] R64A ++++ 127] R134A K134A ++++ [27] K140A ++++ [29] K140A ++++ [29] G143A ++++ [29] I170A ++++ [27] MARV-A GPA38- ND ND 308 -	ZEBOV GP	++++	++++	
ZEBOV GPΔ54-227 ++++ ZEBOV GPΔ54-313 ND ND ZEBOV GPΔ89-149 ++ + ZEBOV GPΔ89-27 ++++ ++++ ZEBOV GPΔ89-213 ND ND D55A ++++ ++++ [27] L57A ++++ ++++ [27] L57A ++++ ++++ [27] L57F ++++ ++++ [27] L53A ++++ ++++ [27] L63A ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R64E ++++ ++++ [27] R64E ++++ ++++ [27] R64A ++++ 127] [27] R64E ++++ [27] [27] R64E ++++ [27] [27] K140A ++++ [29] [170A K140A ++++ [29] [170A MARV-A GPΔ38- ND ND 308 MARV-A	ZEBOV GP∆54-149	++	+	
ZEBOV GPΔ54-313 ND ND ZEBOV GPΔ89-149 ++ + ZEBOV GPΔ89-227 ++++ ++++ ZEBOV GPΔ89-213 ND ND D55A ++++ ++++ [27] L57A ++++ ++++ [27] L57A ++++ ++++ [27] L57F ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R64E ++++ ++++ [27] R64E ++++ ++++ [27] R64A ++++ ++++ [27] R64A ++++ ++++ [27] R64A ++++ 127,28] [30] K95A ++++ +++ [29] K140A ++++ 129] [31] K140A ++++ +++ [29] I170A ++++ +++ [27] MARV-A GP ++++ +++ [27] MARV-A GPA38- + ++++ [27]	ZEBOV GP∆54-227	++++	+++	
ZEBOV GPΔ89-149 ++ + ZEBOV GPΔ89-227 ++++ ++++ ZEBOV GPΔ89-313 ND ND D55A ++++ ++++ [27] L57A ++++ ++++ [27] L57A ++++ ++++ [27] L57F ++++ ++++ [27] L57K ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R58A ++++ +++ [27] R134A ++++ +++ [27] R134A ++++ +++ [29] K140A ++++ 129] [170A K140A ++++ +++ [29] G143A ++++ [29] [170A MARV-A GP ++++ +++ [27] MARV-A GP ++++ [27] [27] MARV-A GPΔ38- + ++++ [27] MARV-A GPΔ60- + +++++ [308 MARV-A GPΔ60- ND <t< td=""><td>ZEBOV GPA54-313</td><td>ND</td><td>ND</td><td></td></t<>	ZEBOV GPA54-313	ND	ND	
ZEBOV GPΔ89-227 ++++ ++++ ZEBOV GPΔ89-313 ND ND D55A ++++ ++++ [27] L57A ++++ ++++ [27] L57A ++++ ++++ [27] L57F ++++ ++++ [27] L53A ++++ ++++ [27] L57K ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R134A ++++ +++ [27] R134A ++++ +++ [29] G143A ++++ +++ [29] G143A ++++ +++ [29] ITOA ++++ +++ [27] MARV-A GP Δ38- + ++++ [27] MARV-A GPΔ38- ND ND 308 MARV-A GPΔ60- + ++++ ++++ 188 - - - MARV-A GPΔ60- ND ND 308 - I41A ++++	ZEBOV GP∆89-149	++	+	
ZEBOV GPΔ89-313 ND ND D55A ++++ ++++ [27] L57A ++++ ++++ [27] L57I ++++ ++++ [27] L57F ++++ ++++ [27] L57K ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R64A ++++ ++++ [29] K140A ++++ ++++ [29] G143A ++++ ++++ [29] I170A ++++ ++++ [27] MARV-A GPΔ38- ND ND 308 MARV-A GPΔ60- + ++++ 188 MARV-A GPΔ60- ND ND 308 L41A ++++	ZEBOV GP∆89-227	++++	++++	
D55A ++++ ++++ [27] L57A ++++ ++++ [27] L57I ++++ ++++ [27] L57F ++++ ++++ [27] L53A ++++ ++++ [27] L63A ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R64A ++++ ++++ [27] R64E ++++ ++++ [29] K140A ++++ ++++ [29] G143A ++++ ++++ [27] MARV-A GPA38- ND ND 308 MARV-A GPA60- + ++++ ++++ 188 - - - MARV-A GPA60- ND ND - 308 -	ZEBOV GP∆89-313	ND	ND	
L57A ++++ ++++ [27] L57I ++++ ++++ [27] L57F ++++ ++++ [27] L57K ++++ ++++ [27] L63A ++++ ++++ [27] L63A ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] F88A ++++ ++++ [27] R134A ++++ +++ [29] K140A ++++ ++++ [29] G143A ++++ ++++ [29] I170A ++++ ++++ [27] MARV-A GP ++++ ++++ [29] I170A ++++ ++++ [27] MARV-A GPA38- + ++++ [27] MARV-A GPA38- ND ND 308 MARV-A GPA60- + +++++ 188 MARV-A GPA60- ND ND 308 I41A ++++ ++++ K18A ++++<	D55A	++++	++++	[27]
L57I ++++ ++++ [27] L57F ++++ ++++ [27] L57K ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R58A ++++ ++++ [27] R134A ++++ +++ [27] R134A ++++ +++ [29] K140A ++++ +++ [29] G143A ++++ +++ [29] I170A ++++ ++++ [27] MARV-A GP ++++ ++++ [27] MARV-A GPA38- + ++++ [27] MARV-A GPA38- ND ND 308 MARV-A GPA38- ND ND 308 MARV-A GPA60- + +++++ 188 MARV-A GPA60- + +++++ 188 MARV-A GPA60- ND ND 308 I41A ++++ +++++ 145 K79A ++++ +++++ 145 <t< td=""><td>L57A</td><td>++++</td><td>++++</td><td>[27]</td></t<>	L57A	++++	++++	[27]
L57F ++++ ++++ [27] L57K ++++ ++++ [27] L63A ++++ ++++ [27] R64E ++++ ++++ [27] R58A ++++ ++++ [27] R134A ++++ +++ [27] R134A ++++ +++ [29] K140A ++++ +++ [29] G143A ++++ +++ [29] I170A ++++ +++ [27] MARV-A GP ++++ 127] [27] MARV-A GPA38- + ++++ [27] MARV-A GPΔ38- + ++++ [27] MARV-A GPΔ38- + ++++ [27] MARV-A GPΔ38- ND ND 308 MARV-A GPΔ60- + ++++ ++++ 188 - - - MARV-A GPΔ60- ND ND 308 - I41A ++++ ++++ - - K18A ++++ ++++ - <t< td=""><td>L57I</td><td>++++</td><td>++++</td><td>[27]</td></t<>	L57I	++++	++++	[27]
L57K ++++ ++++ [27] L63A ++++ +++ [27] R64E ++++ ++++ [27] F88A ++++ +++ [27] F88A ++++ +++ [27] R134A ++++ +++ [29] K140A ++++ +++ [29] G143A ++++ +++ [29] G143A ++++ ++++ [29] I170A ++++ ++++ [27] MARV-A GP ++++ ++++ [27] MARV-A GPA38- + ++++ [27] MARV-A GPA38- ND ND 308 MARV-A GPA38- ND ND 308 I41A ++++ ++++ ++++ 188	L57F	++++	++++	[27]
L63A ++++ +++ [27] R64E ++++ ++++ [27] F88A ++++ +++ [27] K95A ++++ +++ [27] K140A ++++ +++ [29] K140A ++++ +++ [29] G143A ++++ +++ [29] G143A ++++ +++ [29] I170A ++++ ++++ [27] MARV-A GP ++++ 129] [17] MARV-A GPA38- + ++++ [27] MARV-A GPΔ38- ND ND 308 MARV-A GPΔ60- + ++++ ++++ 188 - - - MARV-A GPΔ60- ND ND 308 - L41A ++++ ++++ - - K79A ++++ ++++ - - K118A ++++ ++++ - - K127A ++++ ++++ - - Y146A ND ND <td>L57K</td> <td>++++</td> <td>++++</td> <td>[27]</td>	L57K	++++	++++	[27]
R64E ++++ ++++ [27] F88A ++++ ++ [27,28] K95A ++++ +++ [29] R134A ++++ +++ [29] K140A ++++ +++ [29] G143A ++++ +++ [29] I170A ++++ ++++ [27] MARV-A GP ++++ 129] I170A ++++ ++++ MARV-A GPA38- + ++++ 188	L63A	++++	+++	[27]
F88A ++++ ++ [27,28] K95A ++++ +++ [27] R134A ++++ +++ [29] K140A ++++ +++ [29] G143A ++++ +++ [29] G143A ++++ ++++ [29] I170A ++++ ++++ [27] MARV-A GP ++++ ++++ [27] MARV-A GPA38- + ++++ [27] MARV-A GPA38- ND ND 308 MARV-A GPA60- + ++++ ++++ 188	R64E	++++	++++	[27]
K95A ++++ [27] R134A ++++ [29] K140A ++++ [29] G143A ++++ [29] G143A ++++ [29] I170A ++++ [27] MARV-A GP ++++ [27] MARV-A GPA38- + ++++ 188 - - MARV-A GPA38- ND ND 308 MARV-A GPA38- ND ND - 308 - - - MARV-A GPA60- + ++++ - 188 - - - MARV-A GPA60- ND ND - 308 - - - K118A ++++ ++++ - K118A ++++ ++++ - G127A ++++ ++++ - Y146A ND ND -	F88A	++++	++	[27,28]
R134A ++++ [29] K140A ++++ [29] G143A ++++ !29] I170A ++++ !29] I170A ++++ !21] MARV-A GP ++++ !27] MARV-A GP Δ38- + ++++ 188	K95A	++++	+++	[27]
K140A ++++ [29] G143A ++++ [29] I170A ++++ [29] I170A ++++ [27] MARV-A GP ++++ [27] MARV-A GP A38- + ++++ 188	R134A	++++	++	[29]
G143A ++++ [29] I170A ++++ [27] MARV-A GP ++++ [27] MARV-A GPA38- + ++++ MARV-A GPA38- ND ND 308 - - MARV-A GPA60- + ++++ 188 - - MARV-A GPA60- ND ND 308 - - L41A ++++ ++++ K79A ++++ ++++ K118A ++++ ++++ G127A ++++ ++++ Y146A ND ND	K140A	++++	+++	[29]
1170A ++++ [27] MARV-A GP ++++ ++++ MARV-A GPΔ38- + +++++ 188 ND ND MARV-A GPΔ38- ND ND 308	G143A	++++	+++	[29]
MARV-A GP +++++ +++++ MARV-A GPΔ38- + +++++ 188 - - MARV-A GPΔ38- ND ND 308 - - MARV-A GPΔ60- + +++++ 188 - - MARV-A GPΔ60- + +++++ 188 - - MARV-A GPΔ60- ND ND 308 - - I41A ++++ ++++ K79A ++++ ++++ K118A ++++ ++++ G127A ++++ ++++ Y146A ND ND	I170A	++++	+++	[27]
MARV-A GPΔ38- + +++++ 188 ND ND 308 ND ND MARV-A GPΔ38- ND ND 308 - +++++ MARV-A GPΔ60- + +++++ 188 - - MARV-A GPΔ60- ND ND 308 L41A ++++ ++++ K79A ++++ ++++ K118A ++++ ++++ G127A ++++ ++++ Y146A ND ND	MARV-A GP	++++	++++	
188 MARV-A GPΔ38- 308 ND ND 308	MARV-A GP∆38-	+	++++	
MARV-A GPΔ38- 308 ND ND MARV-A GPΔ60- 188 + +++++ MARV-A GPΔ60- 308 ND ND L41A ++++ ++++ K79A ++++ ++++ K118A ++++ ++++ G127A ++++ ++++ Y146A ND ND	188			
308 MARV-A GPΔ60- + +++++ 188 MARV-A GPΔ60- ND ND 308 - L41A ++++ ++++ K79A ++++ ++++ G127A ++++ ++++ Y146A ND ND	MARV-A GPA38-	ND	ND	
MARV-A GPΔ60- + +++++ 188 - MARV-A GPΔ60- ND ND 308 - L41A ++++ K79A ++++ K118A ++++ G127A ++++ Y146A ND ND ND	308			
188 ND ND 308 ++++ ++++ L41A ++++ ++++ K79A ++++ ++++ G127A ++++ ++++ Y146A ND ND	MARV-A GP∆60-	+	++++	
MARV-A GPΔ60- 308 ND ND L41A ++++ ++++ K79A ++++ ++++ K118A ++++ ++++ G127A ++++ ++++ Y146A ND ND	188			
308 L41A ++++ K79A ++++ K118A ++++ G127A ++++ Y146A ND	MARV-A GP∆60-	ND	ND	
L41A ++++ ++++ K79A ++++ ++++ K118A ++++ ++++ G127A ++++ ++++ Y146A ND ND	308			
K79A ++++ ++++ K118A ++++ ++++ G127A ++++ ++++ Y146A ND ND	L41A	++++	++++	
K118A ++++ ++++ G127A ++++ ++++ Y146A ND ND	K79A	++++	++++	
G127A ++++ ++++ Y146A ND ND	K118A	++++	++++	
Y146A ND ND	G127A	++++	++++	
	Y146A	ND	ND	

++++: >75% of wild-type GP.

+++: 50-75% of wild-type GP.

++: 25-50% of wild-type GP.

+: <25% of wild-type GP.

ND: GP specific bands not detected.

^a Intensities of GP specific bands in the lysate of 293T cells.

^b The ratio between intensities of GP and the VSV M specific band in the supernatant of 293T cells. Download English Version:

https://daneshyari.com/en/article/10764070

Download Persian Version:

https://daneshyari.com/article/10764070

Daneshyari.com